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Full Length Research Article

Evaluating the impact of *Alchornea cordifolia* (Christmas Bush) Root Bark, Seeds and Pod Husks on the Gonads, Serum level of Testosterone, Estrogen, Serum Enzymes and Blood Corpuscles of Rabbits

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Twenty four (5 months old) mixed breeds of rabbits comprising twelve males and females with an average weight of 1.52kg were used for a study to compare the impact of root bark, seeds and pod husk of *Alchornea cordifolia* on the gonads, serum level of testosterone, estrogen, serum enzymes and blood corpuscles of rabbits. Fresh roots and seeds of *A. cordifolia* were harvested and grounded into powders. Five grams each of the different powders (root bark, seed and pod husk) representing treatments B, C and D respectively were administered into one kilogram (l kg) of the concentrate feed (commercial breeders mash) and fed to the animals in their groups—B (root bark), C (seed) and D (pod husk) in a completely randomized design (CRD) experiment. The animals in group A representing control were given only the concentrate feed without any of the A *cordifolia* powder. Results showed that the control (A) recorded the highest feed intake of (3.32 ± 0.39 kg), weight gain and feed efficiency showed no significant difference ($p>0.05$) among the various groups. Treatment C (seed) had the highest weight gain of 1.36 ± 0.72kg followed by treatment A (1.32± 0. 75kg,), treatment B (1.15 ± 0 .55kg) and D (1.09± 0. 72kg). The means of Packed Cell Volume (PCV) and Haemoglobin (Hb) levels were not significantly different ($P>0.05$) between the control and other groups. The results showed a progressive reduction in estrogen and progesterone levels right from the control to the other groups. The lowest productive level of estrogen and progesterone were recorded in treatment C (0.20 n mol/L) and D (9.5nmol/L).

Key words:*Alchornia cordifolia*, Rabbits, Root bark, Pod husk, Seeds, Organs, Feed efficiency

INTRODUCTION

Alchornea cordifolia is one of the plants that grows ubiquitously in the southern Nigeria and used as performance enhancers in livestock and poultry industry (Wekhe and Njoku, 2000). It is also said to provide a state of intense excitement followed by a deep and sometimes fatal depression (Oliver, 1986).Timibitei *et al* (2013) indicated that since the pod husk gave the highest mean testis size of 6.4 ± 3.00, it could be targeted by farmers for increasing the male activity and productivity. Alikwe and Owen (2014) observed that the leaf contain 0.11% cardiac glycosides and 2.4% saponins. Wekhe and Njoku (2000) reported the effect of *Alchornea cordifolia* on gonads and ovary and observed its hypertrophic effect on the gonads which may lead to increase in testosterone production and atrophy of the ovary which may lead to the suppression of estrogen production and related hormones and thus precipitate masculinization and reproductive impotency. Further observation by Wekhe and Njoku, (2000) showed enlarged bursa of fabricus in the female. This was reported to be due to stress reaction. Wekhe, (2002) further buttressed the effect of *Alchornea cordifolia* on the bursa of fabricus as a response of the birds to antigenic effect of the plant in an attempt to get rid of the stress. Testosterone, estrogen and progesterone are one of the gonadotropic hormones that regulate the formation and functioning of the ovary and testis of livestock animals (Guyton and Hall, 1996). Testosterone for instance is responsible for the distinguishing characteristics of the masculine body (Guyton and Hall, 1996). It also has effect on bone growth, calcium retention, muscular development, electrolyte balance, water balance and red blood cells. Estrogen on the other hand, promotes proliferation and growth of specific cells in the body that are responsible for development of most secondary sexual characteristics of the females while progesterone are concerned almost entirely with final preparation of the uterus for pregnancy and breast for lactation (Guyton and Hall,1996).It is therefore envisaged that *Alchornea cordifolia* may exert similar influence like testosterone and other vital physiological or biological activities on rabbits as was observed on broilers by (Wekhe and Njoku.2000). Its response to stress may also be observed since the bursa of fabricus of broilers; a lymphoid organ that responds to stress was provoked (Wekhe and Njoku 2000. and Wekhe. 2002). Wekhe and Njoku, (2000) claimed that *Alchornea cordifolia* increases the size of gonads. The increased gonads were further explained by the same workers to cause increase in testosterone level of broiler birds. They also discovered atrophy of the ovaries of the birds placed on the root bark powder at 1.25g/kg and 2.5g/kg feed. *Alchornea*

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cordifolia also has been shown to cause hypertrophy of the gonads in birds which signifies increased testosterone production, which could bring about increased male activity and reproductivity(Wekhe and Njoku, 2000). Literature on the effects of *Alchornea cordifolia* on the physiological or biological activities in monogastric livestock such as rabbits are scarce. It is therefore the objective of this study to evaluate the impact(s) of root bark, seeds and pod husk of *A. cordifolia* on the serum level of testosterone, estrogen, blood corpuscles (red and white blood cells) and some serum enzymes of rabbits.

MATERIALS AND METHODS

Preparation of Alchornea cordifolia powder

Fresh roots and seeds of *A. cordifolia* were harvested from within Akenfa- Epie in Yenagoa Local Government Area of Bayelsa State. The roots were washed to remove soil and dirt, debarked and the seeds were depoded and all were sun dried separately from the pod husks for four weeks. The sun dried products were grounded into powder sieved bottled and labeled. The proximate composition was done at the Department of Food Sciences and Technology, Rivers State University of Science and Technology (RSUST), Port Harcourt.

Experimental animals and their management

Twenty four (5 months old) rabbits comprising twelve males and females procured from the Livestock Unit, Rivers State Agricultural Development Programme (ADP), Rumuodomaya in Obior/Akpor Local Government Area was used for the study. The rabbits on the average weighed 1.52kg comprising of New Zealand White, Dutch belted and Chinchilla. They were housed in wire mesh cages detached from a single tier hutch with three compartments in a single room (cell) under the same environmental condition of the rabbitary unit of Teaching and Research Farm of RSUST, Port Harcourt. They were properly tagged according to the experimental groups/replicates for proper identification.

Feeding

Five grams each of the different powders (root bark, seed and pod husk) representing treatments B, C and D respectively were administered into one kilogram (l kg) of the concentrate feed (commercial breeders mash) (Tables 1 and 2) and fed to the animals in their groups—B (root bark), C (seed) and D (pod husk). The animals in group A representing control were given only the concentrate feed without any of the A *cordifolia* powder. The animals were given 150g of concentrate each per day and leftovers were subtracted to get their daily feed intakes. The concentrate was supplemented with green forages such as *Panicum maximum* (Guinea grass), *Centrosema pubescens* (centro), *Calapogonuim muconoides* (calapo). Also clean cool water was provided on daily basis. Both feed and water were given *ad libitum* for eight weeks. The feeding, drinking troughs and trays were cleaned and washed on daily basis and also disinfected as often as necessary to ensure proper hygiene.

Medication

The animals were subcutaneously given 0.22mis each of Ivomec injection during the third week of the study. This drug was administered to treat mange infestations, deworm them and also protect them from infection.

Table l. Ingredients and chemical composition of breeder mash

Ingredients	Quantity/100kg
Maize	30
Maize offal	25.00
Soyabean meal	7.00
Groundnut cake	10.0
Wheat bran	14.25
Palm kernel cake	10.00
Bone meal	0.30
Salt	0.30
Premix*	0.25
Lysine	0.10
Methionine	0.10
Total	100.00
Calculated analysis	
ME Kcal/kg	2.60
Crude protein (%)	17.00
Calcium (%)	1.10
Phosphorus (%)	0.80

*The composition of the layers premix is as follows: Vit A, 10,000,0001 U, Vit D3, 2,000,00IU, Vit E, 12,000IU, Vit K, 2g, Thiamine - D|, 1.5g Riboflavin- B2, 5g,Pyradoxine - B6, 1.5g, Vit B12, 10g, Biotin, 20g, Nacine 15g, Panthotenic acid 5g, folic acid 0.5g, Manganese 75g, Zinc 50g, Iron 25g Copper 5g, Iodine ig, Selenium 100g, Cobalt 300g Antioxidant 125g, Choline Chlorine 150g.

Experimental Design

The twenty four rabbits (12 females and 12 males) were randomly divided mto four groups of six animals per group representing one control group and three treatment groups m a completely randomized design as follows: Group A — control Og/kg feed, Group B — Root bark 5g/kg feed, Group C — seed 5g/kg feed and Group D — pod husk 5g/kg feed Each group comprised six replicates (3 males and 3 females) housed individually.

Procedure for Data Collection

At the expiration of the pre-conditioning period, all the rabbits were randomly allocated to the different treatment groups (A-D) were weighed using the Hanna Kitchen scale. This was done to determine the initial body weights of the animals. The animals were subsequently weighed as the study progressed on weekly basis to get their weight gain. Weight gain was obtained by subtracting the initial weight from the total weight (final weight - initial weight = weight gain). This parameter was evaluated by measuring 150g out of the feed from each group (A-C) using the Hanna Kitchen scale. At the end of the day, the left over (remnant) was weighed to determine the difference between the initial quantity (150g) and the quantity consumed by the rabbits in each group/ replicate. Feed efficiency was also calculated by dividing the weight gain with the feed intake weigh (weight gain/feed intake).

Collection of Blood Sample

At the end of the eight weeks of the experiment blood samples were collected from the jugular vein of the sacrificed rabbits (a male and female per group). Two sets of properly labelled sterilized glass tubes containing EDTA (ethylene diamine tetra acetic acid) for determination of haematological parameters;

and a second set of glass tubes without anticoagulant for serum. The sera samples were taken to the University of Port Harcourt Teaching Hospital Laboratory where they were assayed for hormones (testosterone, estrogen and progesterone) and also for enzymes aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphate (ALP). The bottles with EDTA analyzed for total and differential counts of white blood counts (WBC), packed cell volume (PCV) and hemoglobin (Hb) levels. This was done in the River State University of Science and Technology Medical Laboratory. A total of sixteen (16) samples were collected.

Analytical Procedure

Haematological studies

The haematological parameters were determined following the methods outlined in Lamb (1981), and summarized here. The Packed Cell Volume (PCV) was determined by spinning about 75 UL of each blood sample in heparinized capillary tubes in a hematocrit micro centrifuge for 5 min. The Red Blood Cell (RBC) count and White Blood Cell (WBC) count were estimated using normal saline as the diluting fluid. The hemoglobin concentration (HBC) was estimated using cyanomethemoglobin method while the Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and the Mean Corpuscular Volume (MCV) were calculated by Lamb,(1981) procedure. Aspartate transaminase (AST), Alanine transaminase (ALT) and Alkaline phosphatese (ALP) activities were determined using spectrophotometnc methods as described by Rej and Hoder. (1983); McComb, *et al* (1983) respectively.

Statistical Analysis

Data collected were subjected to statistical analysis using SPSS version 17 (SPSS Inc. Chicago). Analysis of variance (ANOVA) was carried out using according to Steel and Torrie. (1980) followed multiple comparison using least significant difference (LSD).

RESULTS AND DISCUSSION

The proximate analysis of the breeders mash fed the rabbits was as follows: crude protein (CP) and metabolizable energy (ME) of 17.0% and 2.60kcal/g respectively. It offered the normal nutrition required for maximum performance. This is in line with the recommended nutrient requirement of breeding rabbits by Fielding (1991) who recommended CP and energy of 15-17 % and 2.60 to 2.70 kcal/g respectively, it therefore presupposes that, whatever effect contrary from maximum performance noticed by the treatment groups comes from the test plant. However, the extra CP and energy of 13.10% and 2.50% respectively recorded in treatment C (seed) may be a pointer to extra performance of the numerical increase of the weight gain of 1.36 ± 0.72kg. The CF and low CP in treatments B and D suggest that these parts of *Alchornea cordifolia* are lower in protein and energy (Table 2). Also the very low energy level of 2.20 kcal/g in treatment D (pod husk) as against treatments B and C may be due to analytical error. Rabbits are pseudo ruminants and as such are able to utilize forages as well as concentrates (Aduku and Olukosi, 1990). This also buttressed the uniqueness of rabbits over other farm livestock such as pigs and poultry (Cheeke *et al,* 1986). The no significant difference (P>0.05) in weight gain of rabbits shows that *Alchornea cordifolia* do not promote weight gain as reported by Wekhe and Njoku (2000).This is in agreement with the findings of Emenalom *et al* (2009) who observed that there was marked deterioration in feed conversion values, body weight and feed intake of broilers fed *Alchornea* seed meal diets. Values for mortality were not significantly different but increased with increasing dietary inclusion level of *Alchornea* seeds in the diets. This might also be ascribed to the no significant differences in feed intake and the age of the animals used as recorded by Fielding (1991). The authors found that the growth rate of rabbits between 5 and 9 months is very slow. The gradual decrease in the feed intake from treatment A - D may suggest that treatment B (root bark) is more palatable than C (seed) and D (Pod husk). The results of the evaluator and comparative impacts of the root bark, seed and pod husk of *A. cordfolia* on rabbits are presented in Table 2.

Table 2. Proximate compositions of the treatment plant and control dietTreatments

Items	A (control)	B (Root bark)	C (Seed)	D (Pod husk)
Dry matter (%)	85.90	84.90	78.50	89.20
Crude protein (%)	17.00	3.50	13.10	6.60
Crude fibre (%)	12.00	6.03	4.03	5.79
Ether extract (%)	3.00	8.90	15.20	4.70
NFE (%)	47.90	57.07	31.37	57.31
Ash (%)	6.00	9.40	7.40	14.80
Energy (kcal/g)	2.60	2.80	2.50	2.20

Table 3. Comparative performance of rabbits to ingestions of A. cordifolia Root bark Seed and Pod husk

Parameters	Treatments			
	A (Control) (Og /kg feed)	B(Root bark) (5g/kg feed)	C(Seed) (5g / kg feed)	D(Pod husk) (5g / kg feed)
Initial weight(kg)	1.51^{b}±0.13	1.54^{a} ±0 18	1.54^{a} ±0. 24	1.50^{b} ±0.05
Final weight(kg)	2.83^{b}±0.47	2.69^{c}±0.62	2.90^{a} ±0.77	2.59^{c}±0.79
Weight gain (kg)	1.32^{a} ±0.37	1.15^{b} ±0. 55	1.36^{a} ± 0 72	1.09^{bc}± 0.72
Feed intake (kg)	3.78^{a} ±0.37	3.76^{a} ±0. 45	3.61^{b}±0. 31	3.32^{c} ± 0.39
Feed efficiency	0.36^{a} ±0.23	0. 33^{b}±0.18	0.38^{a}± 0.19	0.34^{b}± 0.24

The results indicated that there was no significant difference (P > 0.05) in initial weights amongst the four groups (A- D), and also no significant difference (P>0.05) in the feed intake by the rabbits among the treatment groups. The control (A) recorded the highest feed intake of (3.32 ± 0.39 kg). Results on weight gain and feed efficiency also showed no significant difference (p>0.05) among the various groups. Treatment C (seed) had the highest weight gain of 1.36 ± 0.72kg followed by treatment A (1.32± 0. 75kg,), treatment B (1.15 ± 0 .55kg) and D (1.09± 0. 72kg) in this descending order. Results of the haematological parameters are presented in Table 4 and 5. The means of Packed Cell Volume (PCV) and Haemoglobin (Hb) levels were not significantly different (P>0.05) between the control and other groups. Numerical differences were observed in the values of the total White Blood Cell (WBC) and differential counts in the various groups (Table 5). The values of WBC counts were slightly higher in groups B, C and D for both males and females than the control (A). The differential counts of WBC showed fluctuations in the values of neutrophils and lymphocytes. Results did not show the presence of basophils in all the groups. Males had more eosinophils than females in all the groups while monocytes were almost absent.

Table 4. Haematological values of experimental rabbits

		Treatment (Means S.D)				
Parameters	Sex	A (control)	B (Root bark)	C(Seed)	D (Pod husk)	Level of significance
PCV (%)	Male	40±0.00	38±1.41	39.5±0.71	4111.41	n.s
	Female	39±3.00	39±0.71	38±3.00	36±1.41	n.s
Hb (g|dl)	Male	13.15+0.07	12.8±0.00	33.25±0.2	13.4±0.56	n.s
	Female	12.9±0.14	13.1±0.14	32.5±0A4	11.7±0.14	n.s

S.D - Standard Deviation n.s - not significant (P>0.05).

Table 5. Haemotological values of experimental rabbits

Parameters	Sex	A (control)	B (Root bark)	C(Seed)	D (Pod husk)
WBC ($X10^9$/L)	Male	3.5	3.9	4.2	3.8
	Female	3.7	4.0	4.1	4.4
Neutrophils (%)	Male	18	17	42	25
	Female	18	16	22	20
Basophils (%)	Male	-	-	-	-
	Female	-	-	-	-
Eosinophils (%)	Male	2	1	2	4
	Female	1	-	-	-
Monocytes (%)	Male	-	-	-	-
	Female	-	-	-	

Table 6 shows the effect of *A. cordifolia* on some of the reproductive hormones (Estrogen and Progesterone) of the animals. The results showed a progressive reduction in estrogen and progesterone levels right from the control to the other groups. The lowest productive level of estrogen and progesterone were recorded in treatment C (0.20 nmol/L) and D (9.5nmol/L). Treatments B and D showed production level of B (0.30 nmol/L and 10.4nmol/1) and D (0.26nmol/1 and 10.2nmol/) respectively. The result of the Packed Cell Volume (PCV) agrees with the range of 31.0-48.6% as reported by (Mituruka and Rawnsley, 1977). Haemoglobin (Hb) levels were also within reported range of9.9-19.3g /100ml by (Tuffery. 1995). This normal range of Hb suggests an efficient oxygen transport system, which enhances tissue respiration in all the treatment groups (Blood *et al,* 1979). The total white blood cell (WBC) count was also observed to be within normal values of 2.0-15.$Ox10^3$/'ml reported by (Tuffery, 1995). This is an indication that the defensive system of the rabbits in both the control and other treatment groups were not at variance. The neutrophil count of other groups (B-D) was higher than the control (A). The highest was seen in treatment C (seed) with 42% and 22% in both males and females as against 18% in treatment A. These higher values indicate that 5g/kg feed of *Alchornea cordifolia* in B to D did not impose stress on the rabbits.

Table 6. Hormonal value of rabbits (nmol/L)

	Treatments			
Hormones	A (control)	B (Root bark)	C (Seed)	D (pod husk)
Estrogen	0.32	0.30	1.20	0.26
Progesterone	11.00	10.40	9.50	10.20

The increase in number may be due to additional energy and crude protein from the test plant apart from that in the breeders mash. Other haematological components observed were the Basophils, eosinophils and monocytes. The study showed that Basophils and monocytes were completely absent. The absence of these parameters suggests that *Alchornea cordifolia* did not cause chronic inflammatory reactions in the blood system of the rabbits. There were no sharp differences in eosinophils between control and other groups; thus indicating that allergic conditions did not arise from the *Alchornea cordifolia* ingestion of rabbits. The levels of the blood parameters examined were similar in both male and female rabbits and between the control and other groups. This suggest that sex and breed differences may not have a significant role in the haematological blood parameters in this study as observed by Casey *et al,* (1934) to be rather unimportant. It also further suggests that *Alchornea cordifolia* has no effect on the haematological parameters evaluated in both males and females in groups B to D. This report agrees with that of Schermer. (1967) that rabbits do not show any appreciable changes m haematological parameters with changes in nutrition or environment. The importance of hormones in reproduction can never be over emphasized. This is because the entire process of reproduction is controlled by hormones (Hogarth, 1978). Observations in this study revealed a decrease in the production of estrogen and progesterone in the female rabbits in treatments B - D. This suggest the possibility that female rabbits had their ovaries atrophied because of estrogen and progesterone suppression thus leading to masculinization and loss of reproductive potential.

Table 7. Enzyme activities in sera of rabbits (I.U./L)

		Treatment			
Enzyme	Sex	A (control)	B (Root bark)	(Seed)	D (pod husk)
SGOT/AST	Male	99	98	78	89
	Female	79	89	120	90
SGPT/ALT	Male	60	48	60	52
	Female	62	53	68	48
ALP	Male	21	20	17	13
	Female	16	17	20	21

This finding corroborates the observation of Wekhe and Njoku (2000). It was also observed that treatment C (seed) had the lowest level of estrogen and progesterone 0.20 n mol/1 and 9.5 nmol/1 respectively. The lowest ovary weight was also recorded in treatment C (0.16 ± 0.4). The results of the *enzyme* assay shows that values are within maximum threshold of 78.9 and 98.0 i.u./L for AST as recorded by Mitruka and Rawsley (1977). This suggests that protein formation proceeded normally. The higher value of 120 i.u./L in treatment C (seed) may be of no pathological consequence since "there were no signs of disease condition of rabbits in that treatment. This contradicts the report of Keele and Neil (1971) that SGOT are markedly raised in disease and morbid conditions due to injury to large number of metabolically active cells. The slight

variation in values of SGPT/ALT among the groups is usual. The values of ALP showed a sequential decrease from A to D in both males and females. This result revealed that bone formation might be affected if animals were allowed a prolonged intake of *Alchornea cordifolia.* On the other hand, the activities of osteoblasts were not affected by the intake of *Alchornea cordifolia* since the blood level of ALP is usually a good indicator of the rate of bone formation (Guyton. 1991).

Conclusion and Recommendations

From the result of the study, it is recommended that female rabbits should not be fed with *Alchornea cordifolia* since it causes atrophy of the ovaries. It is also recommended that the experiment be repeated with younger growing animals not adults and if adults are used, the period of exposure should not be less than 12 weeks. The level of *Alchornea cordifolia* administered may have been low to produce phenomenal effect needed for the interpretation of the result of this experiment, so the dosage be increased two three fold in future experiment and different inclusion levels of *Alchornea cordifolia*.

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